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ARTICLE

Abnormal lipid profile and hyperinsulinaemia after a mixed meal: additional cardiovascular risk factors in young adults born preterm

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Abstract
Aims/hypothesis Low birthweight in infants born at term is related to the presence of the metabolic syndrome as an adult. Individuals born preterm invariably have low birthweights and may develop the metabolic syndrome as well. Although high BP, glucose intolerance and insulin resistance have been documented, dyslipidaemia has never been reported in individuals born preterm.
Methods In three groups of young adults [29 participants from the POPS (Project On Premature and Small for Gestational Age Infants) cohort born preterm appropriate for gestational age (POPS-AGA), 28 participants from the POPS cohort born preterm small for gestational age (POPS-SGA) and 30 individuals born at term with normal birthweight (CON)] we investigated fasting lipids as well as postprandial responses during a mixed meal test. The relationship between fasting and postprandial measurements and insulin sensitivity, measured by the hyperinsulinaemic clamp, was investigated.
Results Preterm participants had higher BP than CON individuals. Postprandial triacylglycerol levels were increased in POPS-SGA men. POPS-SGA individuals were hyperinsulinaemic during the mixed meal test.
Conclusions/interpretation The mixed meal test provides additional information on cardiovascular risk factors. Postprandial triacylglycerol levels are increased in POPS-SGA men. Postprandial hyperinsulinaemia is found in POPS-SGA individuals.

Keywords Dyslipidaemia · Insulin sensitivity · Mixed meal test · Prematurity

Abbreviations

ATP III	Adult Treatment Panel III of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults
BIA	biometrical impedance analysis
CON	control individuals born at term with normal birthweight
MMT	mixed meal test
POPS	Project on Premature and Small for Gestational Age Infants
POPS-AGA	POPS adult born preterm appropriate for gestational age
POPS-SGA	POPS adult born preterm small for gestational
SDS	standard deviation score
TG	triacylglycerol

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Introduction

In infants born at term, size at birth is related to the presence of the metabolic syndrome [1] throughout adulthood [2–4]. In preterms, size at birth is also reduced and it is becoming clear that preterm infants may be predisposed to the same risks of developing the metabolic syndrome as infants with a low birthweight at term. Insulin resistance has been found in preterm-born prepubertal children [5] and young adults [6]. Glucose intolerance and increased BP are already present in young adulthood, but dyslipidaemia is not found [6, 7].

In the present study the different components of the metabolic syndrome, according to the Adult Treatment Panel III of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) definition, were investigated in young adults born preterm. Because metabolic abnormalities may only become apparent during the postprandial phase, lipid and glucose metabolism were studied after a standardised mixed meal. Furthermore, the hyperinsulinaemic–euglycaemic clamp technique was used to study insulin sensitivity in relation to components of the metabolic syndrome.

Young adults from the Project on Premature and Small for Gestational Age Infants (POPS) born prematurely appropriate for gestational age (POPS-AGA), young adults born prematurely small for gestational age (POPS-SGA) and term-born controls with normal birthweight (CON) were compared.

It was hypothesised that prematurity, especially when accompanied by a low birthweight for gestational age, predisposes for altered lipid metabolism, which can be identified in the postprandial state.

Methods

Participants Participants were recruited from the POPS cohort [8]. The POPS cohort comprises 94% of all Dutch neonates (n=1,338) who were born alive in 1983 with a gestational age of below 32 weeks and/or with a birthweight <1,500 g. Address information of the participants and families was available from the POPS database. Information on disabilities was also available from the database. Disabled individuals were excluded and not approached for this study. Individuals were approached by telephone. Subsequently, an information letter was sent. After 3 weeks individuals were approached again by telephone.

From the POPS cohort we selected 47 individuals born prematurely with an appropriate birthweight for gestational age (birthweight standard deviation score [SDS] between 0 and +2 SDS: POPS-AGA) who had been treated in our Neonatal Intensive Care Unit during the neonatal period. Six individuals (13%) could not be traced, five individuals (11%) were not eligible because of pregnancy and serious disease and four individuals (9%) refused to participate. Thirty POPS-AGA individuals (64%), 15 men and 15 women, were selected after they agreed to participate. Characteristics at birth are shown in Table 1. Of the POPS-AGA group one woman was excluded from the analysis because of serious obesity (BMI 34.6 kg/m^2).

From the POPS cohort we selected 42 individuals born prematurely small for gestational age (birthweight SDS below −2 SDS: POPS-SGA). These participants had been treated in the neonatal period in different centres. One year before the start of our study, 22 of these individuals had participated in a study on renal function. Three individuals (7%) could not be traced, ten individuals (24%) refused to participate. Twenty-eight individuals (67%), 13 men and 15 women, were selected after they agreed to participate. Originally, the definition of SGA in the POPS-SGA group was birthweight SDS below −2 SDS. Because the high refusal rate led to small group size we changed the definition to the group of children within the cohort with the lowest birthweight SDS. Fourteen SGA participants had a birthweight SDS lower than −2 SDS. The range of birthweight SDS between the SGA and AGA group did not overlap. Characteristics at birth are shown in Table 1.

The control group (CON) was recruited by advertisement in the VU University medical faculty. The first 15 men and 15 women, born in 1983, who volunteered for the study were selected. From taking the history we gathered that they were born at term (37–42 weeks) with a birthweight appropriate for gestational age.

Overall, most women (75%) used oral contraceptives. Tests were performed throughout the menstrual cycle, as it was not possible to schedule appointments in the early follicular phase because of logistic reasons.

Study protocol Participants were investigated on two separate days. During the first visit, a physical examination, blood sampling to investigate the presence of the metabolic syndrome and the hyperinsulinaemic–euglycaemic clamp were performed. During the second visit, 2–6 weeks later, a standardised mixed meal test (MMT) was performed.

Physical examination Participants arrived at the examination room after an overnight fast. Measurement of the participant's weight and height were performed using an electronic scale and stadiometer (SECA, Hanover, MD, USA). Weight was measured to the nearest 0.1 kg, height the nearest 0.1 cm. BMI was calculated as weight/height squared and expressed as BMI SDS.

Waist circumference was measured at the level of the umbilicus after full expiration while the individual was standing upright, with feet together and arms hanging freely at the sides, and hip circumference at the level of the greater

Table 1 Clinical characteristics of the POPS-AGA, POPS-SGA and CON groups at birth

	CON	POPS-AGA	POPS-SGA	*p* value[a]
Sex (M/F)	15 M/15 F	15 M/14 F	13 M/15 F	
GA (weeks)	40.0	28.9±1.4	30.7±1.1	<0.0001
Birthweight (g)	3,562±465	1536±300	934±146	<0.0001
Birthweight SDS[b]	0.11±1.0	1.11±0.27	−2.0±0.34	<0.0001

Means±SD
GA, gestational age
[a] Differences between any of the three groups analysed by ANOVA
[b] Birthweight SDS according to Niklasson et al. [23]

trochanter, both with the use of a flexible tape measuring to 0.1 cm accuracy.

Fat mass and the corresponding fat-free mass were estimated by biometrical impedance analysis (AKERN BIA 101/S; RJL Systems, Detroit, MI, USA).

BP was obtained with an automatic BP device (Dinamap; Critikon, Norderstedt, Germany). Three measurements were performed on the non-dominant arm with the individual in a supine position after 15 min of rest, using an appropriate size cuff for arm diameter. Mean values were used in statistical analysis.

Metabolic syndrome components The metabolic syndrome was defined according to the ATP III criteria [9]. Using these criteria, the metabolic syndrome is identified by the presence of three or more of these components: central obesity (waist circumference ≥102 cm [men], >88 cm [women]); raised fasting blood triacylglycerol (TG) (≥1.7 mmol/l); reduced HDL-cholesterol (<1.0 [men] or <1.3 [women] mmol/l); high BP (systolic ≥130 and/or diastolic ≥85 mmHg); and increased fasting glucose levels (≥6.1 mmol/l).

Assays Fasting HDL-cholesterol and TG (HDL-cholesterol: HDL-C Plus, TG: GPO-PAP; Roche, Mannheim, Germany) were measured. Blood glucose was measured immediately by the glucose oxidase method using a Yellow Springs Instrument Glucose Analyzer (Yellow Springs, OH, USA).

Mixed meal test The MMT was performed 2–6 weeks after the clamp study. The participants were investigated after an overnight fast. An i.v. cannula was placed into the antecubital vein for blood sampling. A standardised 400 ml liquid meal (Fresubin Energy; Fresenius Kabi, Friedberg, Germany) with an added 65 ml of unclotted cream was served that contained 76.7 g carbohydrates, 54.7 g fat and 29.3 g proteins.

After an overnight fast from 00:00 hours the previous evening, each participant arrived at 08:30 hours at the test room. An i.v. cannula was placed in an antecubital vein for blood sampling. Fasting blood samples were drawn at −15 and 0 min for measurement of TG, HDL-cholesterol, NEFA, glucose and insulin concentrations.

Thereafter participants were asked to drink the liquid meal within 10 min. Blood collections for the measurement of NEFA (enzymatic colorimetric test, NEFA-C; WAKO Chemicals, Neuss, Germany) and TG levels were performed at 60, 120, 180, 240 and 300 min. Blood collections for the measurement of glucose (hexokinase method; Roche) and insulin (immunometric assay; Bayer Diagnostics, Mijdrecht, the Netherlands) levels were performed at 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min.

Hyperinsulinaemic–euglycaemic clamp After an overnight fast, a hyperinsulinaemic–euglycaemic clamp was performed to determine insulin sensitivity by peripheral glucose uptake as described by DeFronzo et al. [10]. Insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 60 mU kg^{-1} h^{-1} after a priming dose of 6 mU/kg. Hepatic glucose production is known to be suppressed in non-diabetic individuals by this infusion rate. The blood glucose level was measured every 5 min (2300 STATplus C; Yellow Springs). Blood glucose levels were clamped to a level of 5 mmol/l. During the last hour every 15 min blood was drawn to determine plasma insulin concentrations. Euglycaemia (5 mmol/l) was maintained with 20% (wt/vol.) D-glucose infusion. Under steady-state conditions of euglycaemia, the rate of exogenous glucose infusion is equal to the rate of insulin-stimulated glucose disposal. Insulin sensitivity was calculated from the glucose infusion rate (mmol/minute) between 60 and 120 min of the euglycaemic clamp, divided by body weight and expressed as an *M* value (glucose disposal, mmol kg^{-1} min^{-1}) and an *M*i value (glucose disposal, mmol kg^{-1} min^{-1} $[pmol/l]^{-1}$).

Statistical analysis For statistical analysis the SPSS package for Windows version 15 was used (SPSS, Chicago, IL, USA). Results in Tables 1, 2 and 3 are expressed as means ±SD. Differences between the groups and relationships with other factors were tested by linear regression analysis. Differences between groups during the MMT were analysed longitudinally using linear mixed models where time was treated as a categorical variable. With this strategy we were able to analyse the differences between the groups at the

Table 2 Clinical characteristics of the POPS-AGA, POPS-SGA and CON groups at the time of the study

	CON	POPS-AGA	POPS-SGA	CON	POPS-AGA	POPS-SGA	*p* value[a]
Sex (M/F)	M	M	M	F	F	F	
Age (years)	21.7±0.3	22.1±0.4	22.2±0.3	21.50.3	21.9±0.4	22.2±0.3	<0.0001
Height (cm)	185.9±4.8	183.1±8.9	178.9±6.7	171.6±3.8	169.6±7.9	162.3±5.1	0.003
Weight (kg)	76.2±8.2	77.0±16.8	70.3±8.5	63.3±6.5	61.2±9.7	57.9±9.2	0.12
BMI (kg/m^2)	22.0±2.0	22.8±3.4	22.0±3.0	21.5±2.2	21.2±2.1	21.9±2.9	0.94
WC (cm)	77.3±5.6	79.3±9.6	77.7±6.7	69.7±5.3	69.2±4.3	69.7±8.5	0.88
Fat mass (kg)[b]	17.2±4.6	17.9±9.4	15.7±5.5	19.5±4.5	18.5±5.6	18.1±5.3	0.69
Fat percentage[c]	22.2±4.0	21.8±7.1	21.5±5.0	30.4±4.0	29.7±4.6	30.9±4.6	0.81
Fat-free mass (kg)[d]	59.0±4.7	59.2±7.7	55.1±3.0	43.9±2.7	42.7±4.5	39.5±4.6	0.10
M value (mmol kg^{-1} min^{-1})	0.078±0.020	0.057±0.016	0.058±0.021	0.065±0.023	0.051±0.021	0.053±0.012	0.001
*M*i value (mmol kg^{-1} min^{-1} [pmol/l]$^{-1}$)	0.0015±0.0006	0.0010±0.0004	0.0009±0.0004	0.0013±0.0007	0.0010±0.0004	0.0008±0.0003	<0.0001

Results expressed as means±SD
WC, waist circumference
[a] Differences between any of the three groups analysed by ANOVA
[b] Fat mass=weight of body fat derived from BIA measurement
[c] Fat percentage=percentage fat mass derived from BIA measurement
[d] Fat-free mass=weight of body fat-free mass derived from BIA measurement

different time-points in one model. A p value <0.05 was considered to be statistically significant, based on two-sided testing.

Ethical considerations The local ethics committee approved the study. All individuals gave written informed consent to participate.

Results

Clinical characteristics of the groups All participants were born in 1983. POPS-SGA individuals were shorter in comparison with POPS-AGA individuals (p=0.04) and CON individuals (p=0.003) (Table 2).

Metabolic syndrome components Systolic BP was higher in POPS-AGA (p<0.0001) and POPS-SGA (p=0.01) individuals than in CON individuals. No differences were observed between POPS-AGA and POPS-SGA participants (p=0.12). Diastolic BP was higher in POPS-AGA (p=0.001) and POPS-SGA (p=0.007) participants than in CON individuals. No differences were observed between POPS-AGA and POPS-SGA participants (p=0.48) (Table 3).

MMT:TG levels A significant interaction was observed for TG levels with group and sex ($p\leq$0.003 at all time-points from 120 min) (Fig. 1).

TG levels in POPS-SGA men were higher at 120 min (p=0.01), 180 min (p<0.0001), 240 min (p<0.0001) and 300 min (p=0.003) than in CON men. POPS-SGA men had higher TG levels than POPS-SGA women at 120 min (p=0.001), 180 min (p<0.0001), 240 min (p<0.0001) and 300 min (p=0.003).

Among women, no differences were observed between any of the groups.

Table 3 Components of the metabolic syndrome (ATP-III) of the POPS-AGA, POPS-SGA and CON groups during the first visit

	CON		POPS-AGA		POPS-SGA		*p* value[a]
Sex (M/F)	M	F	M	F	M	F	
Fasting glucose (mmol/l)	5.2±0.7	4.8±0.3	5.1±0.3	4.9±0.4	5.3±0.5	5.0±0.3	0.16
HDL-cholesterol (mmol/l)	1.4±0.3	1.6±0.3	1.4±0.2	1.8±0.5	1.4±0.3	1.7±0.4	0.69
TG (mmol/l)	0.9±0.4	1.0±0.4	1.0±0.5	0.8±0.3	1.1±0.5	0.8±0.3	0.94
WC (cm)	77.3±5.6	69.7±5.3	79.3±9.6	69.2±4.3	77.7±6.7	69.7±8.5	0.88
Systolic BP (mmHg)	120±10	116±9	139±15	125±12	130±11	124±14	<0.0001
Diastolic BP (mmHg)	64±5	66±8	73±8	72±5	68±8	73±10	0.002

Results expressed as means±SD
M, male; F, female; WC, waist circumference
[a] Differences between any of the three groups analysed by ANOVA

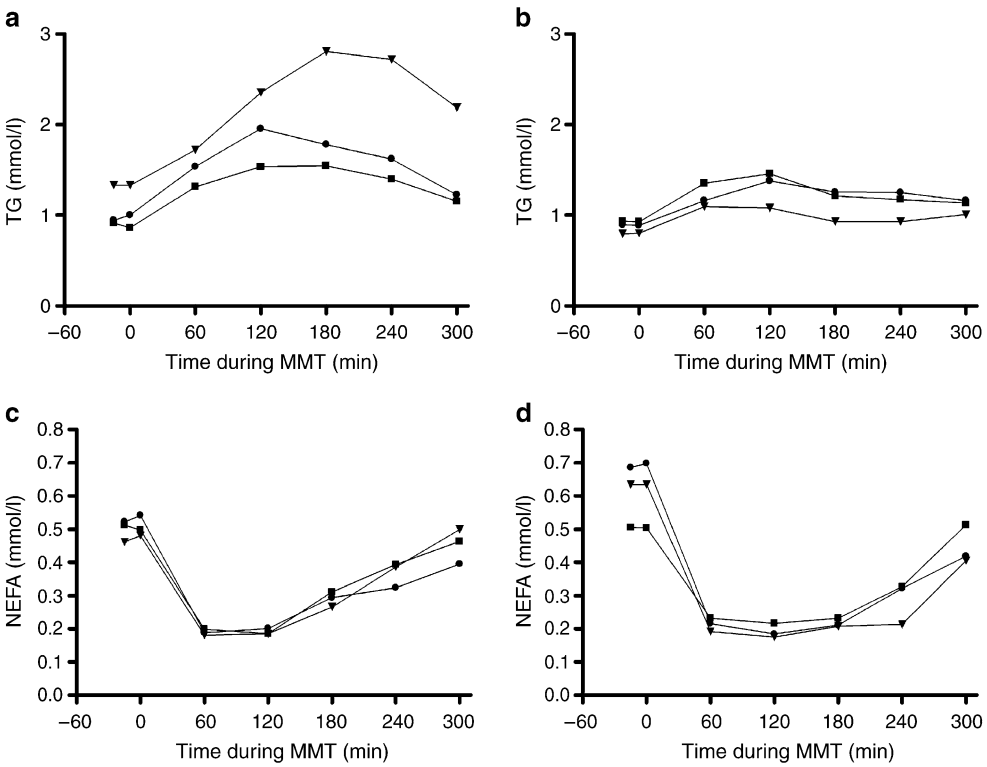

Fig. 1 TG and NEFA levels during MMT in men (**a**, **c**) and women (**b**, **d**) of the CON (squares), POPS-AGA (circles) and POPS-SGA (inverted triangles) groups

MMT:NEFA levels No differences in postprandial NEFA levels were observed between the three groups (Fig. 1).

MMT:glucose levels No differences in postprandial glucose levels were observed between the three groups (Fig. 2).

MMT:insulin levels Insulin levels were higher in POPS-SGA participants compared with CON individuals at 30 min (p=0.002), 60 min (p=0.015), 90 min (p=0.005), 120 min (p=0.001), 150 min (p=0.015), 180 min (p=0.007), 210 min (p<0.0001) and 240 min (p=0.02) (Fig. 2). There

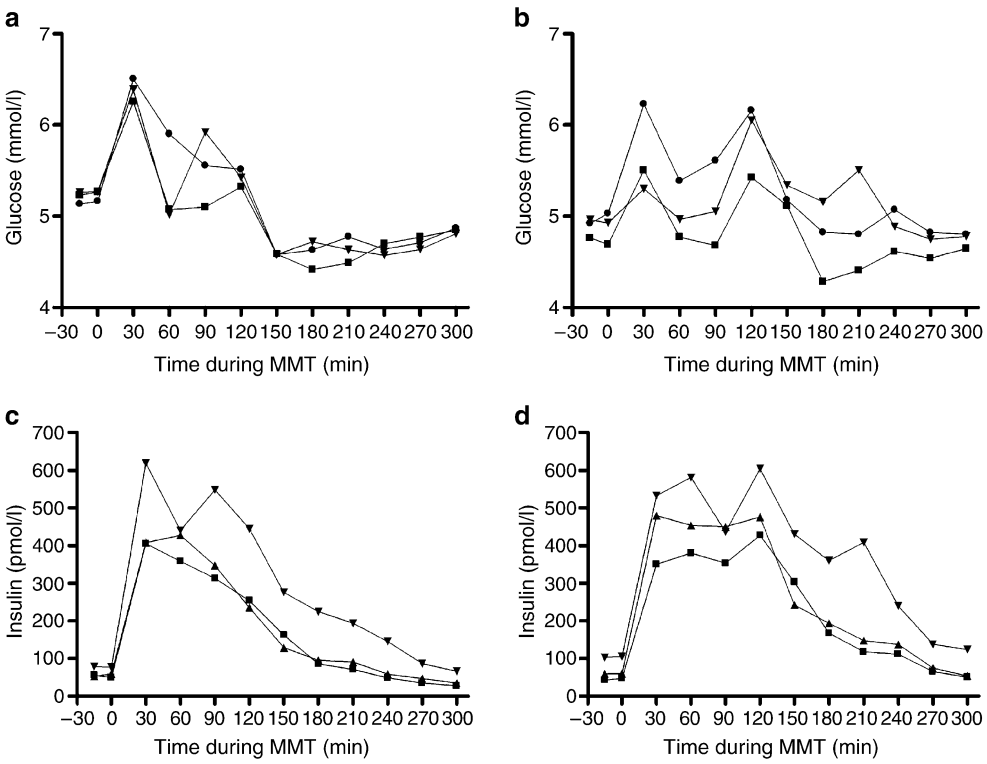

Fig. 2 Glucose and insulin levels during MMT in men (**a**, **c**) and women (**b**, **d**) of the CON (squares), POPS-AGA (upright triangles) and POPS-SGA (inverted triangles) groups

was no difference in insulin levels between CON and POPS-AGA participants.

Insulin sensitivity Insulin sensitivity expressed as an *M*i value was lower in POPS-AGA ($p=0.01$) and POPS-SGA ($p<0.0001$) participants than in CON individuals (Table 2).

Linear regression analysis adjusted for group showed a significant relationship between TG levels and insulin sensitivity at −15 min ($p=0.015$), 0 min ($p=0.007$), 60 min ($p=0.031$) and 300 min ($p=0.019$) during the MMT. The relationship between TG levels and insulin sensitivity was not different between men and women.

Discussion

The metabolic syndrome is a group of cardiovascular risk factors including central obesity, dyslipidaemia, hypertension and raised fasting plasma glucose levels. The development of the metabolic syndrome has been shown to be more prevalent in adults born with low birthweight at term than in control individuals [2, 3]. In individuals born preterm, increased BP, glucose intolerance and insulin resistance have been found [6, 7] but dyslipidaemia was not reported.

As in the previous studies, we did not observe differences in fasting lipid levels between the three groups. However, early signs of metabolic derangement may be more easily detected in the postprandial state. Our study shows that POPS-SGA men, especially, have increased TG levels in the postprandial state.

As in previous reports [11] we found a relationship between insulin sensitivity and TG levels, in which we observed no sex differences.

A weakness of this study is that not enough individuals could be included according to the SGA definition of birthweight less than −2 SDS. Instead, individuals from the cohort with the lowest birthweight had to be selected. Therefore the POPS-SGA group may reflect the effects of both moderate and extreme intrauterine growth retardation, which makes a comparison with the POPS-AGA participants more difficult.

Postprandial hypertriacylglycerolaemia is considered to be a part of the metabolic syndrome [12]. It has been shown that postprandial TG levels are related to the development of cardiovascular disease [13] and that these levels are related to insulin resistance [11]. A gradual rise in TG levels throughout the day with peak concentrations between dinner and bedtime has been reported in individuals with insulin resistance [12]. Potentiation of TG levels may occur in our individuals as well, as we observed maximum postprandial TG levels hours after ingestion of the meal. It seems unlikely that the increased lipid levels that we found are explained by genetic causes of dyslipidaemia because HDL- and LDL-cholesterol were not different between subgroups.

In women, we found consistently lower TG levels throughout the MMT compared with men. A problem of our study is that, because of logistic reasons, the clamp study could not be performed during the follicular phase. Furthermore, 75% of women used oral contraceptives, which are known to influence the metabolic measurements. Previous studies have shown that women have lower postprandial TG levels [14], probably because of the presence of oestrogens and progestagens [15–18].

The MMT has been used for the assessment of beta cell function [19–21]. The oral load results in a postprandial exposure of the pancreas to glucose, amino acids and hormones that closely reflects the ability of the pancreas to produce insulin under normal physiological conditions [19]. We found that postprandial insulin levels were increased in POPS-SGA participants compared with CON individuals. This finding is different from the results of the hyperinsulinaemic clamp study that showed that both POPS-AGA and POPS-SGA participants are more insulin resistant than the CON group. This may be explained by earlier findings suggesting that insulin sensitivity measurements as determined by the clamp and hyperinsulinaemia may identify different metabolic subgroups [22]. Increased insulin levels may also represent compensatory beta cell activity in reaction to insulin resistance and increase the risk of beta cell decompensation and the subsequent development of glucose intolerance and type 2 diabetes in the future.

In conclusion, additional cardiovascular risk markers have been identified in individuals born preterm. POPS-SGA men have increased postprandial TG levels that are related to the degree of insulin resistance. POPS-SGA individuals have increased postprandial insulin levels because of their insulin resistance.

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Duality of interest The authors declare that they have no duality of interest.

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